PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 15/00, C12Q 1/00, A61K 37/00, C07K 7/00, 13/00

A1

(11) International Publication Number: WO 95/24474

(43) International Publication Date: 14 September 1995 (14.09.95)

(21) International Application Number: PCT/US94/05292

(22) International Filing Date: 12 May 1994 (12.05.94)

(30) Priority Data:
08/209,214 10 March 1994 (10.03.94) US

(71) Applicant: HUMAN GENOME SCIENCES, INC. [US/US]; Suite 300, 9620 Medical Center Drive, Rockville, MD 20850-3338 (US).

(72) Inventors: HE, Wei, Wu; 6225 Free Stone Court, Columbia, MD 21045 (US). COLEMAN, Timothy, A.; 7512 Boxberry Terrace, Gaithersburg, MD 20878 (US). HASTINGS, Gregg, A.; Apartment H, 9913 Gable Ridge Terrace, Rockville, MD 20850 (US). ROSEN, Craig, A.; 22400 Rolling Hill Road, Laytonsville, MD 20882 (US).

(74) Agents: OLSTEIN, Elliot, M. et al.; Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068 (US).

(81) Designated States: AU, CA, CN, JP, KR, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: BONE MORPHOGENIC PROTEIN-10

(57) Abstract

The present invention discloses a human bone morphogenic protein (BMP-10) polypeptide and DNA (RNA) encoding such polypeptide. The human BMP-10 polypeptide may be produced by recombinant DNA techniques and is useful in inducing de novo bone formation. Also disclosed is an antibody against such polypeptide, which may be used diagnostically to detect lung cancer.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

BONE MORPHOGENIC PROTEIN-10

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptide of the present invention is Bone Morphogenic Protein-10 (BMP-10). The invention also relates to inhibiting the action of such polypeptides.

There are approximately 600,000 nonunion bone fractures a year in the United States alone. BMP may be used to induce bone and/or cartilage formation and is therefore useful in wound healing and tissue repair. BMP may be used for treating a number of bone defects and periodontal disease and various types of wounds.

A 32-36 kDa osteogenic protein purified from bovine bone matrix is composed of dimers of two members of the transforming growth factor-beta super family, the bovine equivalent of human osteogenic protein-1 and bone morphogenic protein-2a. It is reported that recombinant human osteogenic protein-1 (HOP-1) induces new bone formation *in vivo* with a specific activity compatible with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation *in vitro* (Sampath, T.K., et al., J. Biol.

Chem., 267:20352-62 (1992)). The recombinant human osteogenic protein-1 (HOP-1) was produced in mammalian cells as a processed mature disulfide-linked homodimer with an apparent molecular weight of 36,000. The evaluation of HOP-1 effects on cell growths and collagen synthesis in rat osteoblast-enriched bone cell cultures showed that both cell proliferation and collagen synthesis were stimulated in a dose-dependent manner and increased three-fold in response to 40ng of HOP-1/ml.

It has also recently been shown that ectopic expression of DVR-4 (Bone Morphogenetic Protein-4) induces amphibian embryos to develop with an overall posterior and/or ventral character, and that DVR-4 induces ventral types of mesoderm in animal explants. DVR-4 is therefore the first molecule reported both to induce posteroventral mesoderm and to counteract dorsalizing signals such as activin, (Jones, C.M. et al, Development, 115:639-47 (1992)).

In accordance with one aspect of the present invention, there is provided a novel mature polypeptide which is BMP-10, as well as fragments, analogs and derivatives thereof. The polypeptide of the present invention is of human origin.

In accordance with another aspect of the present invention, there are provided polynucleotides (DNA or RNA) which encode such polypeptides.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptides by recombinant techniques.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides for therapeutic purposes, for example, for the promotion of *de novo* bone formation during surgical insertion of protheses, for the treatment of non-union bone fractures, and for treatment of osteoporosis and periodontal disease.

In accordance with yet a further aspect of the present invention, there is provided an antibody against such polypeptides. Such antibodies may be used diagnostically in the detection of lung disorders by measuring the serum level of BMP-10 in patients.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

FIG. 1a depicts the cDNA sequence and corresponding deduced amino acid sequence of the mature BMP-10 polypeptide. The amino acid sequence is represented by the standard three letter code for amino acids.

FIG. 1b shows the homology between the amino acid sequence of the active domain of BMP-10 (upper line) with that of BMP-3a (lower line).

FIG. 2 depicts the results of a Northern Blot Analysis which indicates the expression levels of the mRNA transcript for BMP-10 in human tissues.

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptide having the deduced amino acid sequence of Figure 1a or for the mature polypeptide encoded by the cDNA of the clone deposited as ATCC Deposit No. 75672 on February 9, 1994.

A polynucleotide encoding a polypeptide of the present invention was discovered in a fetal lung cDNA library. It contains an open reading frame encoding a mature polypeptide of 119 amino acids and shows 80 % sequence identity to the BMP-3a gene product. The polypeptide is a member of the bone morphogenic protein family which is a subfamily of the transforming growth factor Beta (TGF-β) superfamily.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in Figure 1a or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same, mature polypeptide as the DNA of Figure 1a or the deposited cDNA.

The polynucleotide which encodes for the mature polypeptide of Figure 1a or for the mature polypeptide encoded by the deposited cDNA may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of Figure 1a or the polypeptide encoded by the cDNA of the deposited clone. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in Figure 1a or

the same mature polypeptide encoded by the cDNA of the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of Figure 1a or the polypeptide encoded by the cDNA of the deposited clone. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figure 1a or of the coding sequence of the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The present invention also includes polynucleotides, wherein the coding sequence for the mature polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotide of the present invention may encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of Figure 1a or the deposited cDNA.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by

reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to a BMP-10 polypeptide which has the deduced amino acid sequence of Figure 1a or which has the amino acid sequence encoded by the deposited cDNA, as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of Figure 1a or that encoded by the deposited cDNA, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of Figure 1a or that encoded by the deposited cDNA may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments,

derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the BMP-10 genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant

techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or

control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila and Sf9; animal cells such as CHO, COS or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus,

and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early

promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a

selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. It is preferred to have low concentrations (approximately 0.15-5 mM) of calcium ion present during purification. (Price et al., J. Biol. Chem., 244:917 (1969)). Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The BMP-10 may be employed to promote de novo bone formation which may be used in the treatment of periodontal disease and other bone defects of the oral cavity.

BMP-10 may also be used during surgical insertion of protheses. In hip replacements, knee replacements and other surgical insertion of protheses, the prothesis is held in place by surgical cement. The cement eventually loosens, however, making it necessary to perform another surgery. This second surgery is a much more difficult procedure and is responsible for surgeons reluctantance to insert protheses in young people. BMP-10 may, therefore, be used to coat the prothesis before insertion which results in bone formation

around the prothesis, making a stronger union and allowing for the use of less cement.

BMP-10 may also be employed in the treatment of osteoporosis, which is characterized by excessive bone resorption resulting in thin and brittle bones. BMP-10 would stimulate bone formation to help alleviate this condition.

The polypeptides may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

The polypeptides of the present invention may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the polypeptide, and a pharmaceutically

acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides of the present invention may be employed in conjunction with other therapeutic compounds.

BMP-10 is preferably used topically, however, when it is used systemically, the pharmaceutical compositions may be administered in a convenient manner such as by the oral, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, or intradermal routes. The amounts and dosage regimens of pharmaceutical compositions of BMP-10 administered to a subject will depend on a number of factors such as the mode of administration, the nature of the condition being treated, the body weight of the subject being treated and the judgment of the prescribing physician. Generally speaking, pharmaceutical compositions of BMP-10 are given, for example, in appropriate doses of at least about 10 μg/kg body weight and in most cases will not be administered in an amount in excess of about 8 mg/kg body weight, and preferably is given in doses of about 10 μg/kg body weight to about 1 mg/kg daily, taking into account the routes of administration, symptoms, etc.

Specifically, BMP-10 may be prepared as a gel matrix formulation and administered, for example, ectopically, which is preferably administered at the site of bone fracture at a dosage of 50 mg, by applying the matrix

directly to the site of the fracture. This matrix may also be applied to protheses before insertion.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the cDNA is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clones to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This

technique can be used with cDNA as short as 500 or 600 bases; however, clones larger than 2,000 bp have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. FISH requires use of the clones from which the EST was derived, and the longer the better. For example, 2,000 bp is good, 4,000 is better, and more than 4,000 is probably not necessary to get good results a reasonable percentage of the time. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR

based on that cDNA sequence. Ultimately, complete sequencing of genes from several individuals is required to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce

single chain antibodies to immunogenic polypeptide products of this invention.

Antibodies specific to the polypeptide of the present invention may further be used a potential marker for lung cancer. BMP-10 is a protein which is secreted and predominantly expressed in adult lung (Figure 2). Accordingly, antibodies against BMP-10 can be used as part of an *in vitro* diagnostic assay to measure the expression level of this protein in adult subjects. The procedure would consist of drawing blood from a patient, adding the above-mentioned antibodies to the blood serum, then performing a standard immunoassay such as ELISA or a radioimmunoassay to detect the amount of antibody bound to BMP-10 which is directly related to the amount of BMP-10 produced in the lung. Tumor cells are more active than cells with a normal physiology and therefore, produce more BMP-10 which would be indicative of lung cancer.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain

sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of Human BMP-10

The DNA sequence encoding for human BMP-10 is initially amplified using PCR oligonucleotide primers corresponding to the 5' and 3' end of the DNA sequence to synthesize insertion fragments. The 5' oligonucleotide primer 5'-GATCGGATCCAAAGCCCGGAGGAAGCAG-3' contains a Bam HI restriction enzyme site followed by 18 nucleotides of BMP-10 coding sequence starting from amino acid 4 (Lys); the 3' sequence 5'-GTACTCTAGATCACCGGCAGGCACAGGTG-3' contains complementary sequences to an Xba I site, a translation stop codon and the last 16 nucleotides of BMP-10 coding sequence. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc., 9259 Eton Ave., Chatsworth, CA 91311, Catalog No. 33093). The plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His) and restriction enzyme cloning sites. The pQE-9 vector was digested with Bam HI and Xba I and the insertion fragments were then ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture was then used to transform the *E. coli* strain M15/rep4 available from Qiagen under the trademark m15/rep4. M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates containing both Amp and Kan. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 μg/ml) and Kan (25 μg/ml). The O/N culture is used to inoculate a large culture at a ratio of

1:100 to 1:250. The cells were grown to an optical density of 600 ($O.D.^{600}$) between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 2-4 hours. Cells were then harvested by centrifugation (20 mins. at 6000Xg). The cell pellet was solubilized in the chaotropic agent 6 molar Guanidine HCl. After clarification, solubilized BMP-10 was purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag. (Hochuli, E. et al., Genetic Engineering, Principles & Methods, 12:87-98 (1990). BMP-10 (95% pure) was eluted from the column in 6 molar GHCl pH 5.0. Protein renaturation out of GHCl can be accomplished by several protocols. (Jaenicke, R. and Rudolph R., Protein Structure - A Practical Approach, IRL Press, New York (1990). The pH 5.0 eluate was diluted and reapplied to a second nickel-chelate column. Bound protein was renatured by running a linear descending gradient of GnHCl. This allows for folding to occur on the column. The protein was then eluted with 250 μm imidazole pH 5.0.

Example 2

Tissue Distribution of BMP-10

Northern blot analysis was carried out to examine the levels of expression of BMP-10 in human tissues. Total cellular RNA samples were isolated with RNAzol™ B system (Biotecx Laboratories, Inc., 6023 South Loop East, Houston, TX 77033). About 10 ug of total RNA isolated from each human tissue specified was separated on 1% agarose gel and blotted onto a nylon filter (Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989)). The labeling reaction was done according to the Stratagene Prime-It kit with 50 ng DNA fragment. The labeled DNA was purified with a Select-G-50 column from 5' Prime -- 3 Prime, Inc.,

5603 Arapahoe Road, Boulder, CO 80303. The filter was then hybridized with radioactive labeled full length BMP-10 gene at 1,000,000 cpm/ml in 0.5 M $NaPo_4$ and 7% SDS overnight at 65°C. The filters were washed twice at room temperature and twice at 60°C with 0.5 x SSC, 0.1% SDS, the filters were then exposed at -70°C overnight with intensifying screen. The message RNA for BMP-10 is abundant in lung. Figure 2.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HE, ET AL.

(ii) TITLE OF INVENTION: Bone Morphogenic Protein - 10

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CARELLA, BYRNE, BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN
(B) STREET: 6 BECKER FARM ROAD
(C) CITY: ROSELAND
(D) STATE: NEW JERSEY
(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/209,214
(B) FILING DATE: 10 MARCH 1994
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: FERRARO, GREGORY D.
(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-140

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 201-994-1700
(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 360 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
AAGACGATTC AGAAAGCCCG GAGGAAGCAG TGGGATGAGC CGAGGGTGTT CTCCCGGAGG  60
TACCTGAAGG TGGACTTCGC AGACATCGGC TGGAATGAAT GGATAATCTC ACCGAAATCT 120
TTTGATGCCT ACTACTGCGC GGGAGCATGT GAGTTCCCCA TGCCTAAGAT CGTTCGTCCA 180
TCCAACCATC CCACCATCCA GAGCATTGTC AGGGCTGTGG GCATCATCCC TGGCATCCCA 240
GAGCCCTGCT GTGTTCCCGA TAAGATGAAC TCCCTTGGGG TCCTCTTCCT GGATGAGAAT 300
CGGAATGTGG TTCTGAAGGT GTACCCCAAC ATGTCCGTGG ACACCTGTGC CTGCCGGTGA 360
```

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 119 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS:
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Lys Thr Ile Gln Lys Ala Arg Arg Lys Gln Trp Asp Glu Pro Arg
5 10 15
Val Phe Ser Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly
20 25 30
Trp Asn Glu Trp Ile Ilc Ser Pro Lys Ser Phe Asp Ala Tyr Tyr
35 40 45
Cys Ala Gly Ala Cys Glu Phe Pro Met Pro Lys Ile Val Arg Pro
50 55 60
Ser Asn His Ala Thr Ile Gln Ser Ile Val Arg Ala Val Gly Ile
65 70 75
Ile Pro Gly Ile Pro Glu Pro Cys Cys Val Pro Asp Lys Met Asn
80 85 90
Ser Leu Gly Val Leu Phe Leu Asp Glu Asn Arg Asn Val Val Leu
95 100 105
Lys Val Tyr Pro Asn Met Ser Val Asp Thr Cys Ala Cys Arg
110 115

WHAT IS CLAIMED IS:

1. An isolated polynucleotide encoding for BMP-10, said polynucleotide selected from the group consisting of

(a) a polynucleotide encoding for the BMP-10, polypeptide having the deduced amino acid sequence of Figure 1a or an fragment, analog or derivative of said polypeptide;

(b) a polynucleotide encoding for the BMP-10 polypeptide having the amino acid sequence encoded by the cDNA contained in ATCC Deposit No. 75672 or an fragment, analog or derivative of said polypeptide.

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. The polynucleotide of Claim 2 wherein said polynucleotide encodes for BMP-10 having the deduced amino acid sequence of Figure 1a.

6. The polynucleotide of Claim 2 wherein said polynucleotide encodes for the BMP-10 polypeptide encoded by the cDNA of ATCC Deposit No. 75672.

7. The polynucleotide of Claim 1 having the coding sequence for BMP-10 as shown in Figure 1a.

8. The polynucleotide of Claim 2 having the coding sequence for BMP-10 deposited as ATCC Deposit No. 75672.

9. A vector containing the DNA of Claim 2.

10. A host cell genetically engineered with the vector of Claim 9.

11. A process for producing a polypeptide comprising: expressing from the host cell of Claim 10 the polypeptide encoded by said DNA.

12. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 9.

13. An isolated DNA hybridizable to the DNA of Claim 2 and encoding a polypeptide having BMP-10 activity.

14. A polypeptide selected from the group consisting of (i) a BMP-10 polypeptide having the deduced amino acid sequence of Figure 1a and fragments, analogs and derivatives thereof and (ii) a BMP-10 polypeptide encoded by the cDNA of ATCC Deposit No. 75672 and fragments, analogs and derivatives of said polypeptide.

15. The polypeptide of Claim 14 wherein the polypeptide is BMP-10 having the deduced amino acid sequence of Figure 1a.

16. An antibody against the polypeptide of claim 14.

17. A method for the treatment of a patient having need of BMP-10 comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 14.

18. A method for the detection of a lung abnormality in a patient comprising:

drawing a blood sample from the patient;
separating the blood serum from the blood sample; adding to the blood serum an effective amount of the antibody of claim 16;

performing an immunoassay; and

determining the concentration of BMP-10 in the blood serum.

19. A pharmaceutical composition comprising the polypeptide of Claim 14 and a pharmaceutically acceptable carrier.

20. The method of Claim 17 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

DEPICTS THE AMINO ACID AND DNA SEQUENCE OF THE BMP -10. FIG. 1A

```
   1  AAGACGATTCAGAAAGCCCGGAGGAAGCAGTGGGATGAGCCGAGGGTGTTCTCCCGGAGG
      LysThrIleGlnLysAlaArgArgLysGlnTrpAspGluProArgValPheSerArgArg

  61  TACCTGAAGGTGGACTTCGCAGACATCGGCTGGAATGAATGGATAATCTCACCGAAATCT
      TyrLeuLysValAspPheAlaAspIleGlyTrpAsnGluTrpIleIleSerProLysSer

 121  TTTGATGCCTACTACTGCGCGGGAGCATGTGAGTTCCCCATGCCTAAGATCGTTCGTCCA
      PhrAspAlaTyrTyrCysAlaGlyAlaCysGluPheProMetProLysIleValArgPro

 181  TCCAACCATGCCACCATCCAGAGCATTGTCAGGGCTGTGGGCATCATCCCTGGCATCCCA
      SerAsnHisAlaThrIleGlnSerIleValArgAlaValGlyIleIleProGlyIlePro

 241  GAGCCCTGCTGTGTTCCCGATAAGATGAACTCCCTTGGGGTCCTCTTCTCGGATGAGAAT
      GluProCysCysValProAspLysMetAsnSerLeuGlyValLeuPheLeuAspGluAsn

 301  CGGAATGTGGTTCTGAAGGTGTACCCCAACATGTCCGTCCACACCTCTGCCTGCCGGTGA
      ArgAsnValValLeuLysValTyrProAsnMetSerValAspThrCysAlaCysArgEnd
```

SUBSTITUTE SHEET (RULE 26)

1/2

FIG. 1B

BMP10/BMP3A ACTIVE DOMAIN AMINO ACID ALIGNMENT

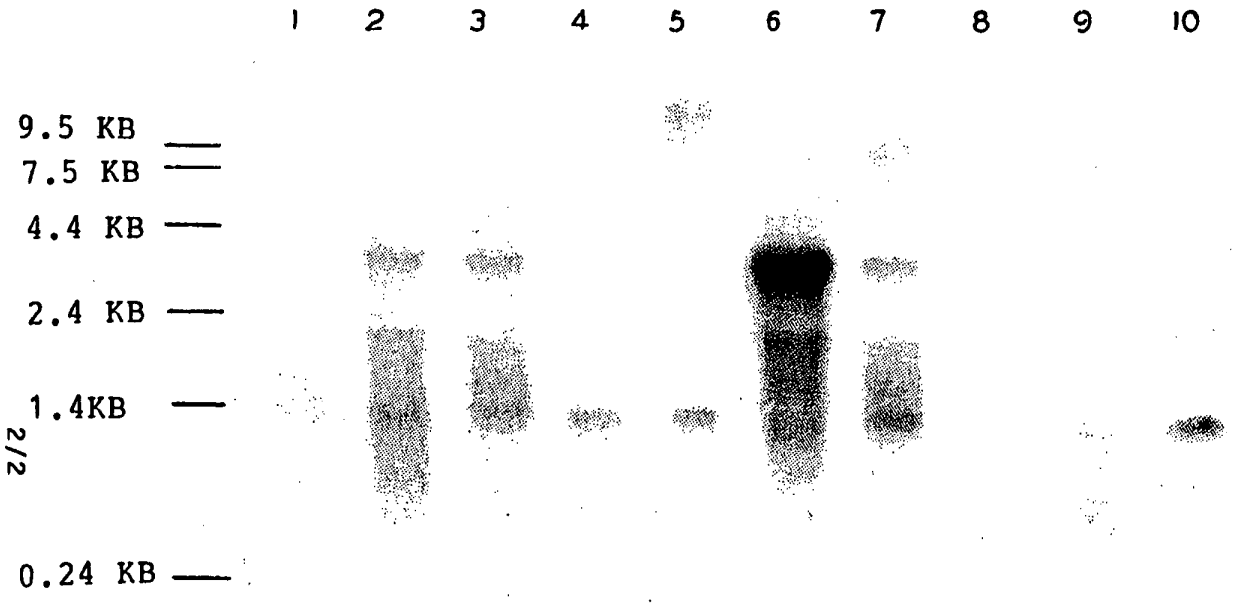
```
HMP10      1 KTIQKARRKQWDEPRVFSRRYLKVDFADIGWNEWIISPKSFDAYYCAGACEFPMPKIVRP 60
             +T++KARRKQW EPR  +RRYLKVDFADIGW+EWIISPKSFDAYYC+GAC+FPMPK ++P
BMP3A    354 QTLKKARRKQWIEPRMCARRYLKVDFADIGWSEWIISPKSFDAYYCSGACQFPMPKSLKP 413

BMP10     61 SNHATIQSIVRAVGIIPGIPEPCCVPDKMNSLGVLFLDENRNVVLKVYPNMSVDTCACR 119
             SNHATIQSIVRAVG++PGIPEPCCVP+KM+SL +LF VEN+NVVLKVYPNM+V++CACR
BMP3a    414 SNHATIQSIVRAVGVVPGIPETCCVPEKMSSLSILFFDENKNVVLKVYPNMTVESCACR 472
```

WO 95/24474

PCT/US94/05292

FIG. 2



NORTHERN BLOT ANALYSIS OF BMP-10

Lane 1 = Ovary
Lane 2 = Testes
Lane 3 = Gall Bladder
Lane 4 = Kidney
Lane 5 = Liver
Lane 6 = Lung
Lane 7 = Spleen
Lane 8 = Prostate
Lane 9 = Hippocampus
Lane 10 = Heart

INTERNATIONAL SEARCH REPORT

In tional application No.
PCT/US94/05292

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 15/00; C12Q 1/00; A61K 37/00; C07K 7/00, 13/00

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 69.1, 172.3, 240.2, 252.3, 320.1; 514/2, 12, 44; 530/324, 350, 387.1 ; 536/23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155, 351, 357, 358) search terms: BMP-3, BMP-10, bone morphogenetic protein, osteogenic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. CELL SCI. SUPPL., Volume 13, issued 1990, Wozney et al, "Growth factors influencing bone development", pages 149-156, see entire document.	1-20
A	MOLECULAR REPRODUCTION AND DEVELOPMENT, Volume 32, issued 1992, Wozney et al, "The Bone Morphogenetic Protein Family and Osteogenesis", pages 160-167, see entire document.	1-20
X	US, A, 5,116,738 (WANG ET AL) 26 May 1992, see figures 1-2.	1-2, 9-14, 19
A	US, A, 5,284,756 (GRINNA ET AL) 08 February 1994, see figures, abstract, claims.	1-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 July 1994	JUL 28 1994

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Marianne Porta Allen Jill Warden for
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

In. ational application No. PCT/US94/05292

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,168,050 (HAMMONDS, JR. ET AL) 01 December 1992, see column 3, lines 22-40, and Example 1.	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05292

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Telephone Practice
Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

In- ational application No.
PCT/US94/05292

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 7.1, 69.1, 172.3, 240.2, 252.3, 320.1; 514/2, 12, 44; 530/324, 350, 387.1 ; 536/23.5

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-15 and 19, drawn to nucleotide sequences, vectors, host cells, process to make protein, process to make cells, protein, and pharmaceutical composition, classified in at least Class 536, subclass 23.5, for example.

II. Claims 16 and 18, drawn to antibodies and methods of detection using antibodies, classified in at least Class 530, subclass 387.1, for example.

III. Claims 17 and 20, drawn to gene therapy, classified in at least 514, subclass 44.

The claims of groups I-III are drawn to distinct inventions and are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept. The claims of group I form a single inventive concept.